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(54) Title: A COMPOSITION BASED ON NATURAL EXTRACTS USEFUL IN THE PREVENTION AND TREATMENT OF CUTANEOUS WRINKLES

(57) Abstract: A composition based on natural extracts useful in the prevention and treatment of cutaneous ageing and particularly wrinkles, which comprises in combination: leucocyanadines in the form of extract of *Vitis vinifera*; triterpenes in the form of an extract of *Centella asiatica*; fish cartilage extract.

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A composition based on natural extracts useful in the prevention and treatment of cutaneous wrinkles

The present invention relates to a composition based on natural extracts, intended for oral administration, for the therapeutic and/or cosmetic treatment of skin ageing phenomena, and in particular wrinkles.

Among the main objectives of modern dermatology is, without doubt, the etiopathogenetic understanding of that cutaneous phenomenon generally indicated with the term wrinkle.

Wrinkles - like cellulite and alopecia - are among the most unwelcome of unsightly characteristics, and are considered as the most characteristic indication of the transition from youth to old age.

From a pathogenetic point of view it is considered that the causes responsible for the formation of wrinkles may be:

- 1) chronological cutaneous ageing generally, in large part supported by the action of free radicals of oxygen;
- 2) degradation of the elastic fibres and alteration of collagen fibres by phenomena principally induced by the action of the metallo-protease released by keratinocytes and fibroblasts in response to exposure to light;
- 3) muscular and articular movements; and
- 4) the force of gravity.

These four elements act in different amounts in the formation of wrinkles, giving rise to different types.

Currently wrinkles are classified thus:

- network or fine mesh lines;

- expression or muscle-following lines;
- articulation lines;
- muscular-cutaneous relaxation folds; and
- position folds.

Of these the only wrinkles which can respond to treatment (peeling and surgery apart) are the cutaneous network lines and expression lines. These latter, in fact, already well defined by about twenty to thirty years, can become very deep and then become rather evident in subjects with chronic photo damage, independently of age. In this case, the expression lines become more marked because of the worsening relationship of the thicker and more rigid corneal layer, and of the thinner epidermis.

Consequently, rehydrating the skin, protecting it from ultra violet rays and - above all - stimulation with suitable substances, can obtain a partial reduction in the depth of the expression lines. Even better results can be obtained in the treatment of cutaneous network lines: these latter become ever more evident with the passage of the years and exposure to light and can appear and become very evident even starting from thirty to forty years.

The prevention of the said cutaneous ageing phenomena has been up to now based essentially on the use of high protection solar filters.

There are also known compositions for topical use, which use as active agents, retinoid and/or α -hydroxy acid derivatives.

The present invention provides an active, systemic, composition capable of preventing the formation of new wrinkles (particularly of network and expression lines) and to reduce and resist the aggravation of those already existing.

The invention also provide a composition useful for the treatment of skin stretch marks and skin relaxations.

It constitutes therefore an object of the invention to provide a composition based on natural extracts, useful in the above-mentioned treatments, characterised in that it comprises, in combination:

- leucocyanidines in the form of an extract of *Vitis vinifera*;
- triterpenes in the form of an extract of *Centella asiatica*; and
- fish cartilage extract.

In particular, the extract of leucocianidine or leucoanthocianine of *Vitis vinifera* comprises procianidolic oligomers, typically dimers, trimers, tetramers, pentamers and heptamers, derived from the condensation of monomeric units of flavan-3-ols and flavan-3, 4-diols free or esterified or gallic acid.

Typically, the commercial extracts of leucocyanidine are categorised in terms of their content of catechine and epicatechine which constitute the principal components thereof.

The principal active components in the commercial extracts have good bioavailability when orally administered; within

the scope of the invention dry extracts can therefore be utilised as such. However, the use of phytosomal forms is preferable, which further improves the bioavailability of the active principle. In this form, leucocyanidines are complexed with phospholipids, particularly with soya distearoylphosphatidylcholine.

The triterpene fraction of *Centella asiatica* is obtained by extraction and purification of the aerial part of the *Centella asiatica*, also known as asiatic Hydrocotyl. Typically, this triterpene fraction comprises a mixture of madecassic acid (about 30% wt) asiatic acid (about 30% wt) and asiaticoside (about 40% wt).

These substances, with well known toxicological profile, recognise the fibroblast as principle target and perform their functions by interacting with it. It has been shown that these triterpenes accelerate the uptake and metabolism of lysine and proline, the two fundamental amino acids in the final structure of collagen and increase the synthesis and release of tropocollagens, likewise stimulating the turnover of the mucopolysaccharide acids in the connective tissue, consequently preserving the functionality of the connective matrix.

Within the scope of the present invention the triterpenes of *Centella asiatica* can be utilised in the form of dry extracts or, preferably, in phytosomal form that is - as it has been mentioned - in a form complexed with phospholipids for improving the absorption thereof by the organism.

The extracts of fish cartilage, used in the scope of the invention, comprise as active principle, chondroitin sulphate

constituted by a mixture of mucopolysaccharide acids, formed by a repetitive linear unit containing different sulphate groups. The basic units are constituted by N-acetyl-galattosamine and glucuronic acid.

The use of anhydrous extract of shark cartilage is particularly preferred.

Tests conducted by the applicant have made it possible to ascertain that the association of the active principles mentioned above performs an effective anti oxidant action acting to limit the cutaneous damage caused by free radicals, an anti collagenasic and anti elastasic action directed at limiting the damage consequent upon the release of those metalloproteases involved in the photo exposure inflammatory cascade after photo exposure which is the source of those connective scars responsible in time for the cutaneous sinking identified as wrinkles, a procollagenogenetic action directed at reconstructing the pool of connective collagen destroyed by the protease released following photo exposure and an excellent tropism for the connective and cutaneous tissue.

In a preferred embodiment the basic composition according the invention comprises:

- 5-200 parts by weight of extract of *Centella asiatica*, preferably in phytosomal form;
- 10-300 parts by weight of extract of leucocyanadine, preferably in phytosomal form; and
- 50-1000 parts by weight of shark cartilage.

Commercially available standardised extracts can be utilised for the preparation of the composition.

In the case of compositions intended for subjects who are smokers, it is preferable that the composition contains, in association with the said active principles, also lycopene, which consists of a carotenoid acyclic lipophilic extract from the skin of tomato free from "provitamin A" type activity which has a powerful antioxidant activity. Lycopene can be typically utilised in quantities from 0.1 percent by weight to 1 percent by weight referred to 100 parts by weight in total of the basic mixture, constituted by the above mentioned components.

Particularly in the case of compositions intended for the treatment of wrinkles in menopausal women it is preferable that the composition contains - in association with the said active principles - soya isoflavones; for this purpose commercial extracts of soya isoflavones can be utilised, preferably in quantities from 20-60% by weight with reference to 100 parts by weight of the basic composition constituted by the extracts of *Centella asiatica*, leucocyanadine and fish (shark) cartilages.

Compositions for use on men, particularly for male subjects of an age greater than 40-45 years, further preferably comprise as active principle, the hormone dehydroepiandrosterone (DHEA) which can be introduced in to the formulation by means of the use of Dioscorea or steroid extract of YAM. The DHEA as such is preferably utilised in quantities from 5-40% - preferably from 5-20% by weight with reference to 100 parts by weight of the above mentioned basic mixture.

In a preferred embodiment the composition further includes dimeric flavones of *Ginkgo Biloba*, preferably in the form of

phytosomes in which the active principles are complexed with phospholipids (particularly diastearoylphosphatidylcoline).

The said dimeric flavones are introduced into the composition by utilising extracts of *Ginkgo biloba* highly enriched in biflavonic component.

Five biflavones have been identified in particular in the biflavonic component of *Ginkgo biloba*, namely amentoflavone, bilobethine, isoginkgetine, ginkgetine and sciadopisine. Within the scope of the present invention the activity of the said dimeric flavones in the prevention and treatment of cutaneous ageing has been ascertained, presumably due to their micro vascular-kinetic properties. Their use is particularly useful for the treatment of cutaneous ageing in smokers.

The extracts of dimeric flavones are preferably used in the compositions in quantities from 2-30%; more preferably from 5-20% by weight referred to 100 parts by weight of the basic mixtures.

The composition can moreover include active principles chosen from eicosapentaenoic acid (EPA), docahexaenoic acid (DHA), γ -linolenic acid and their mixtures. Fish oil is a preferred source of eicosapentaenoic acid (EPA) and docahexaenoic acid (DHA) which, with reference to 100 parts by weight of the basic mixture, can be added in the amount of 5-80 percent by weight.

The γ -linolenic acid is preferably introduced into the formulation by the use of borage oil added in quantities from 30-80 percent by weight referred to 100 parts by weight of

the basic mixture. Alternatively, or in combination with borage oil, the invention contemplates the use of oil of evening primrose (eonoothera) in equal quantities.

Vitamins, particularly vitamin E, vitamin C and β -carotene can also be included in the compositions, as can micronutrients and mineral salts as solids, zinc and/or selenium.

The composition according to the invention is formulated in a form suitable for oral administration, in particular capsules of soft or hard gelatine shells, tablets, pills, elixirs, suspensions and syrups.

The forms of administration can include excipients and/or binders and/or pharmaceutically acceptable vehicles, in particular lecithin and mono and diglycerides of fatty acids.

By way of example, a typical formulation can be formulated according to the data shown in the following table where, for the components of the basic mixture and for the optional components there are indicated the minimum and maximum quantities preferred, expressed in parts by weight.

Components Of Basic Mixture	A Parts By Weight (min)	%	B Parts By Weight (max)	%
Cartilage	50		1000	
Leucocyanidine (extract)	10		300	
<i>Centella asiatica</i> (extract)	20		200	
	80	100	1500	100

Optional Components	C Parts By Weight (min)	C/A *100	D Parts By Weight (max)	D/B *100
Isoflavones (soya extract)	48	60	600	40
Dimeric flavones	10	12.5	50	3.3
Lycopene	0.10	0.125	10	0.1667
DHEA (as such)	15	18.75	150	10
Vitamin E	10	12.50	200	13.33
Zinc as ions	5	6.25	15	1.0
Borage oil or EPO (oenothera)	50	62.50	1000	66.7
Fish Oil	50	62.50	750	50

In the above table the values shown, expressed in parts by weight, when expressed in mg correspond to minimum and maximum advised daily dosages.

The efficacy of the composition according to the invention has been determined by means of studies effected of female subjects free from cutaneous and/or systemic pathologies. Capsules have been utilised with cases of soft gelatine containing:

- extract of *Centella asiatica*: 20 mg/cps
- extract of leucocyanadine: 50 mg/cps
- shark cartilage: 250 mg/cps
- soya lecithin: 50 mg/cps
- fish oil: 120 mg/cps
- borage oil: 240 mg/cps
- Vitamin E: 15 mg/cps
- Zinc: 5 mg/cps.

The subjects took three capsules per day of the product under examination for ninety days and the results obtained were

compared with the results of a corresponding number of subjects to when a placebo was administered.

Evaluation of the photo ageing was effected by measurement by the following biophysical parameters: cutaneous thickness by ultrasound at 20 MHz, biomechanical functions of the skin in particular elasticity, stretchability, viscoelasticity and micro circulation by Doppler laser velocimeter and evaluation of the depth of the wrinkles, performed by the technique of cutaneous replication with image analysis.

Whilst the subjects treated with placebo exhibited no significant changes, in the group treated with the active principles there was detected a significant increase in the microcirculatory values, cutaneous thickness and bio mechanical function of the skin both in terms of stretchability viscoelasticity and elasticity.

Moreover, analysis of the images of the skin replicas showed a reduction in the depth of the wrinkles with particular reference to the deepest wrinkles and to the undulations of the skin.

The compositions, moreover, have been found useful for reducing skin stretch marks and cutaneous relaxations.

CLAIMS

1. A composition based on natural extracts useful in the prevention and treatment of cutaneous ageing, skin stretch marks and skin relaxations, characterised in that it comprises, in combination:

- leucocyanadines in the form of extract of *Vitis vinifera*;
- triterpenes in the form of extract of *Centella asiatica*; and
- extract of fish cartilage.

2. A composition according to Claim 1, characterised in that it comprises:

- from 5-200 parts by weight of extract *Centella asiatica*;
- from 10-300 parts by weight of leucocyanidine extract; and
- from 50-1000 parts by weight of fish cartilage.

3. A composition according to claim 2, characterised in that it further includes one or more of the following components:

- from 48-600 parts by weight of soya extract containing isoflavones;
- from 0.1-10 parts by weight of lycopene;
- from 15-150 parts by weight of dehydroepiandrosterone;
- from 50-1000 parts by weight of borage oil or oil of evening primrose;
- from 10-50 parts by weight of extracts of dimeric flavones; and
- from 50-750 parts by weight of fish oil.

4. A composition according to Claim 1 or Claim 2, characterised in that it comprises, with reference to 100 parts by weight of the basic mixture constituted by the extracts listed in Claim 1:

- from 0.1-1 percent by weight of lycopene.

5. A composition according to any of Claims from 1 to 4, particularly for the treatment of cutaneous ageing in menopausal female subjects, characterised in that it further includes:

- extract of soya containing isoflavones in a quantity from 20-60% by weight referred to 100 parts by weight of the basic mixture of Claim 1.

6. A composition according to any of Claims from 1 to 5, particularly for the treatment of cutaneous ageing phenomena in male subjects, characterised in that it further comprises:

- from 5-40 percent by weight of dehydroepiandrosterone from extract of YAM (dioscorea) with reference to 100 parts by weight of the basic mixture of Claim 1.

7. A composition according to any of Claims 1 to 6, characterised in that it further comprises at least one component chosen from fish oil, borage oil, oil of evening primrose (oenothera), vitamins, micro nutrients and salts of zinc.

8. A composition according to any of Claims from 1 to 7, characterised in that the said extract of *Centella asiatica* is in phytosomal form.

9. A composition according to any of Claims from 1 to 8, characterised in that the said leucocyanadine extract is in phytosomal form.
10. A composition according to any preceding Claim in the form of a dosage unit for oral administration.
11. A composition according to any of Claims from 1 to 10, in which the extract of fish cartilage is an extract of shark cartilage.
12. A composition based on natural extracts useful in the prevention and treatment of cutaneous ageing, particularly wrinkles, characterised in that it comprises, as active principles in association, procyanidolic oligomers of *Vitis vinifera*, chondroitin sulphate, and at least one component selected from the group which consists of madecassic acid, asiatic acid and asiaticoside or a mixture of the said three components.
13. A composition according to Claim 12, characterised in that it comprises in association at least one component selected from the group which consists of lycopene, isoflavones of soya and dehydroepiandrosterone (DHEA).
14. The use of a composition according to any preceding Claim, for the treatment and/or prevention of cutaneous ageing, stretch marks or skin relaxations.

INTERNATIONAL SEARCH REPORT

International Application No

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A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K7/48 A61K35/60 A61K35/78

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 33494 A (KOSBAB JOHN V) 6 August 1998 (1998-08-06) page 7, line 15 -page 11, line 11 page 21, line 31 -page 22, line 10 page 25, line 6 -page 26, line 21 page 27, line 23 - line 26 page 35, last paragraph; claims; table 4	1-14
A	DATABASE PROMT 'Online! STN INTERNATIONAL; AN 1999:21826, 1999 SIMPSON, LIZ: "Supplementary benefits" XP002165003 the whole document & BEAUTY COUNTER; ISSN: 0960-3751, December 1998 (1998-12), page 12 --- -/--	1-14

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

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- *G* document member of the same patent family

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BE 904 014 A (SCHIFANO CARMELO) 2 May 1986 (1986-05-02) page 3, line 11 - line 26 ---	1-14
A	FR 1 433 383 A (LABORATOIRES LAROCHE NAVARRON) 13 June 1966 (1966-06-13) the whole document ---	1-14
A	WO 96 23512 A (AETERNA INC LAB) 8 August 1996 (1996-08-08) claims 53-73 -----	1-14

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/10276

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9833494 A	06-08-1998	AU 6141498 A EP 1021177 A	25-08-1998 26-07-2000
BE 904014 A	02-05-1986	NONE	
FR 1433383 A	13-06-1966	BE 744799 A	01-07-1970
WO 9623512 A	08-08-1996	US 5618925 A AU 719118 B AU 2300195 A AU 717978 B AU 3738895 A BG 62954 B BG 100941 A BG 101870 A BR 9507552 A BR 9510540 A CA 2188793 A CA 2212010 A WO 9532722 A CZ 9702452 A CZ 9603141 A EP 0759765 A EP 0806960 A FI 964327 A FI 973195 A HU 76554 A HU 77488 A JP 9512563 T JP 11502514 T NO 964547 A NO 973564 A NZ 284407 A NZ 294553 A PL 317076 A PL 321678 A RO 114868 B US 6025334 A US 5985839 A	08-04-1997 04-05-2000 21-12-1995 06-04-2000 21-08-1996 29-12-2000 31-07-1997 30-04-1998 05-08-1997 09-06-1998 07-12-1995 08-08-1996 07-12-1995 14-01-1998 16-04-1997 05-03-1997 19-11-1997 20-12-1996 01-10-1997 29-09-1997 28-05-1998 16-12-1997 02-03-1999 30-12-1996 01-10-1997 27-04-1998 29-07-1999 03-03-1997 22-12-1997 30-08-1999 15-02-2000 16-11-1999